

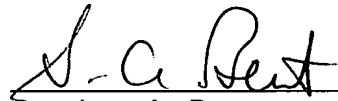
**REMARKS**

Applicants submit this Amendment to indicate the insertion point for the substitute Sequence Listing filed concurrently herewith. Applicants respectfully request examination on the merits of this application.

Receipt of the initial Office Action on the merits is awaited.

Respectfully submitted,

14 January 2002  
Date

  
Stephen A. Bent  
Reg. No. 29,768

**FOLEY & LARDNER**

3000 K Street, N.W., Suite 500  
Washington, D.C. 20007-5109  
Telephone: (202) 672-5300  
Facsimile: (202)672-5399

**Versions with Markings to Show Changes Made**

**IN THE SPECIFICATION**

Please replace the paragraph beginning on page 2 at lines 23 with the following rewritten paragraph:

Hsp73 is a prominent, constitutively expressed, cytosolic stress protein of the Hsp70 family. Among many other known functions, this Hsp mediates selective degradation of cytosolic proteins in an endolysosomal compartment (reviewed in Dice, J.F. and S.R. Terlecky. 1994. Selective degradation of cytosolic proteins by lysosomes. In Cellular proteolytic systems. A.J. Ciechanover and A.L. Schwartz, editors. Wiley-Liss, 55-64; Hayes, S.A. and J.F. Dice, *J. Cell Biol.* 132 (1996):255). It specifically recognizes (SEQ ID NO: 11) KFERQ-like peptide sequences and targets the captured proteins for lysosomal degradation in response to various stimuli (Chiang, H.-L., S.R. Terlecky, C.P. Plant, and J.F. Dice, *Science* 246 (1989):382; Dice, J.F., *Trends Biochem.Sci.* 15 (1990):305; Terlecky, S.R., H.-L. Chiang, T.S. Olson, and J.F. Dice, *J.Biol.Chem.* 267 (1992):9202). Hsp73-associated proteins directly cross the membrane bilayer to enter lysosomes (Dice, J.F. and S.R. Terlecky. 1994. Selective degradation of cytosolic proteins by lysosomes. In Cellular proteolytic systems. A.J. Ciechanover and A.L. Schwartz, editors. Wiley-Liss, 55-64; Terlecky, S.R., H.-L. Chiang, T.S. Olson, and J.F. Dice, *J.Biol.Chem.* 267 (1992):9202). The transport of Hsp73-associated proteins into lysosomes has been reconstituted *in vitro* using highly purified lysosomes. Uptake is stimulated by ATP, requires the stress protein Hsp73, is selective and saturable, and does not involve a vesicular pathway. The lysosomal membrane glycoprotein LGP96 has been identified as a receptor for the import of proteins into lysosomes (Terlecky, S.R., H.-L. Chiang, T.S. Olson, and J.F. Dice, *J.Biol.Chem.* 267 (1992):9202; Cuervo, A.M. and J.F. Dice, *Science* 273 (1996):501). Hsp73 mediates protein import not exclusively into lysosomes but also into endosomal compartments.

Please replace the paragraph beginning on page 10 at line 4 with the following rewritten paragraph:

The term "nuclear localisation signal", as used in accordance with the present invention denotes an amino acid motif within a (poly)peptide which directs the transport of said (poly)peptide into the nucleus of a cell (see, e.g., Stryer L. (ed.), Biochemistry, 1995, W.H. Freeman and Company, New York). Different kinds of nuclear localisation signals (NLS) are known to the person skilled in the art and may, e.g., contain five consecutive positively charged amino acid residues like the SV40 large T antigen NLS: (SEQ ID NO: 1) Lys-Lys-Lys-Arg-Lys.

Please replace the paragraph beginning on page 11 at line 1 with the following rewritten paragraph:

The incorporation of a protease cleavage site between said first and second (poly)peptide is especially useful if said first (poly)peptide is the desired end product. Thus, after synthesis of the fusion protein said second (poly)peptide may be easily separated from said first (poly)peptide by cleaving said protease cleavage site with an appropriate protease. For example, an appropriate protease cleavage site may comprise the amino acid sequence (SEQ ID NO: 2) Lys-Asp-Asp-Asp-Asp-Lys which is specifically recognized by bovine enterokinase.

Please replace the paragraph beginning on page 25 at lines 6 with the following rewritten paragraph:

Vectors used for transfection of cell lines: The BMGneo vector system was used for the generation of stable antigen-expressing transfectants in which genes are expressed under methallothionin promoter control (Karasuyama, H. and F. Melchers, *Eur.J.Immunol.* 18 (1988):97). The wtT-Ag of SV40, the

mutant cytoplasmic cT-Ag variant (with a deletion of SV40 nucleotide position 4490-4392, *i.e.* amino acid position 110-152 of the T-Ag), or the N-terminal T1-272 fragment (T<sub>1-272</sub>) were cloned into the BMG*neo* vector as described (Schirmbeck, R., J. Zerrahn, A. Kuhröber, E. Kury, W. Deppert, and J. Reimann, *Eur.J.Immunol.* 22 (1992):759; Schirmbeck, R., W. Deppert, E. Kury, and J. Reimann, *Cell.Immunol.* 149 (1993):444; Schirmbeck, R., J. Zerrahn, A. Kuhröber, W. Deppert, and J. Reimann, *Eur.J.Immunol.* 23 (1993):1528). The BMG/T411-708 construct was generated from the T-Ag-encoding plasmid pEARLY generously provided by Drs. W. Deppert and V. von Hoyningen (Hamburg, Germany) (von Hoyningen-Huene, V., M. Kurth, and W. Deppert, *Virology* 190 (1992):155). The T411-708-encoding NsiI/BamHI fragment of pEARLY was cloned into the PstI/BamHI site of pBluescript. The resulting plasmid pBlueT411-708 was cut with XhoI and BamHI and cloned into XhoI/BamHI site of the BMG*neo* vector generating the plasmid BMG/T411-708. The BMG/LS construct was generated from plasmid pTKTHBV2 (a generous gift of Dr. M. Meyer, Munich, Germany) and the HBsAg-encoding BMG/S vector (Schirmbeck, R., K. Melber, A. Kuhröber, Z.A. Janowicz, and J. Reimann, *J.Immunol.* 152 (1994):1110; Schirmbeck, R., K. Melber, T. Mertens, and J. Reimann, *J.Virol.* 68 (1994):1418). The preS1/preS2-encoding BglII/XbaI-fragment of HBV, subtype ayw, was cloned into the BglII/XbaI site of pBluescript. The resulting plasmid preS/Blue was cut with XhoI and cloned into the XhoI site of BMG/S yielding the plasmid BMG/LS. The vector BMG/cT1-272RT132 was generated from a cT-Ag encoding plasmid cyBlue and a 400 bp EcoRI-fragment encoding the aa sequence 281-412 of SIVmac239 polymerase (a generous gift of Drs. H. Petry, Göttingen and K. Melber, Düsseldorf). The EcoRI-fragment was cloned into EcoRI site of pBluescript generating the plasmid RT132Blue. This plasmid was cut with HindIII/BamHI to obtain the RT132-encoding 400 bp fragment that was coned into the HindIII/BclII site of cyBlue. The resulting plasmid was linearized with HindIII and fused with a cT1-272-encoding HindIII-fragment form cyBlue to generate the plasmid cT1-272RT132.

The cT1-272RT132 plasmid was cut with XhoI/BamHI and cloned into XhoI/BamHI cut BMGneo yielding the plasmid BMG/cT1-272RT132, which encodes the following amino acid sequence (see Fig. 1D): SV40: cT1-272 ; spacer: (SEQ ID NO: 8) DIEF ; SIVpol281-412 sequence (SEQ ID NO: 3) MLIDFRELNRPVTQDFTEVQLG!PHPAGLAKRKRTVLDIGDAYFSIPLDEEFROYTAFTL PSVNNAEPGKRYIYKVLPOGWKGSPAIFQYTMRHVLEPFRKANPDVTLVQYMDDILI ASDRTDLEHDRVVL; stop: (SEQ ID NO: 9) DPGGS. The spacer and stop sequences were not related to T-Ag or pol. The HBVpreS- encoding sequence was generated from the plasmid preS/Blue (see above) by PCR using primers (SEQ ID NO: 4) 5' TCGA**ATG**GGGCAGAATCTTTCCAC 3' and (SEQ ID NO: 5) 3' CCCTGGGACGCGACTTG**ATT**TCGA 5'. The corresponding start and stop signals of the preS antigen are indicated. The PCR product was cloned into EcoRV-cut pBluescript yielding the plasmid preSstop/Blue. The plasmid preSstop/Blue was digested with Sall and the 5' ends were filled with Klenow polymerase followed by KpnI digestion. A cT1-272 encoding KpnI / SmaI fragment was cloned into the KpnI-SmaI/blunt preSstop/Blue vector generating the plasmid cT1-272preS/Blue. The plasmid cT1-272preS/Blue was cut with Sall and the 5' ends were filled with Klenow polymerase, digested with SmaI and cloned into the XhoI-cut and blunted BMGneo vector yielding plasmid BMG/cT1-272preS. This encodes the following amino acid sequence (see Fig. 1D): SV40: cT1-272 ; spacer: (SEQ ID NO: 10) DIEFLQPSTVSISLIR ; the HBV preS: (SEQ ID NO: 6) MGQNLSTSNPLGFFPDHQLDPAFRANTANPDWDFNPNKDTWPDAANKVGA GAFGLGFTPPHGGLLGWSPQAQGILQTLPANPPPASTNRQSGRQPTPLSPPLRNTHP QAMQWNSTTFHQTLQDPRVRGLYFPAGGSSSGTVNPVLTTASPLSSIFSRIQDPALN . The spacer sequence was not related to T-Ag or preS.

Please replace the paragraph beginning on page 28 at line 18 with the following rewritten paragraph:

**Example 3: Western blotting, and determination of serum antibody levels.**

Western blotting: After SDS-PAGE, gels were incubated for 10 min in equilibration buffer [0.1% SDS, 20mM Tris-acetate (pH8.3)]. Proteins were electroblotted with a Transblot apparatus onto nitrocellulose paper at 60 V for 2 hours. The transfer buffer was 0.1% SDS, 20% isopropanol and 20 mM Tris-acetate (pH 8.3). Nitrocellulose sheets were incubated for 30 min with 50% isopropanol, for 15 min in TBS buffer [150mM NaCl, 5 mM NaN<sub>3</sub>, 1mM EDTA, 15 mM Tris-hydrochloride (pH 7.8)] and for 12 h in buffer G [TBS buffer + 0.1% gelatine + 100µg/ml immunoglobulin-free bovine serum albumin]. Nitrocellulose sheets were washed for 1 h with buffer GT [TBS buffer + 0.1% gelatine + 0.1% Tween 20]. The following polyclonal rabbit antisera were used: anti-T-Ag antiserum, anti- preS1 antiserum, anti-S antiserum, and anti-RT serum (generated against 200 µg SIVpol377-394 (SEQ ID NO: 7) EPFRKANPDVTLVQYMDD peptide and 20 µg ovalbumin in 250 µl PBS, emulsified in 250 µl montanide™). Sheets were incubated for 4-6 h in GT buffer containing 1:500 diluted rabbit antisera. Alternatively, sheets were incubated for 2-3 h with murine mAbs (1:500) (i.e. anti- T-Ag mAb PAb 108, or anti- hsp 70 mAb 3A3 (cat.no. MA3-006; Dianova, Hamburg, Germany), or sera from immunized mice (1:50 - 1: 500), washed and incubated for a further 2 h with 1:200 diluted rabbit anti-mouse antibodies in GT buffer (a generous gift of Dr. W. Deppert, Hamburg, Germany). Unbound antibodies were removed by washes in GT buffer. Thereafter, sheets were incubated for 2-12 h with 0.5-1 µCi of <sup>35</sup>S-labeled protein A (Amersham, Braunschweig, Germany), washed, dried, soaked in 20 % 2,5-diphenyloxazolol (PPO) in toluol and again dried. Radiolabeled immune complexes were detected by fluorography.